RESPONSE TO OFFICE ACTION

A. Status of the Claims

Claims 2-4 and 67 are currently pending and under consideration in this case. Claims 2-3 are amended to delete reference to claim 68. No new matter is added. Claims 2-4 and 67 are presented herein for reconsideration.

B. Rejection Under 35 U.S.C. §112, 2nd Paragraph

The Action rejects claims 2-4 under 35 U.S.C. § 112, 2nd paragraph, as indefinite, for depending on cancelled claim 68. In view of the amendment of claims 2-3, the rejection is believed moot, and its withdrawal is respectfully requested.

C. Rejection Under 35 U.S.C. §103

The Action rejects claims 2-4 and 67 under 35 U.S.C. § 103(a) as obvious over Tomes (U.S. Patent No. 6,258,999), in view of Martin (U.S. Patent 5,057,419), and further in view of Thompson *et al.* (U.S. Patent 6,117,677). Applicants respectfully traverse.

1. The Teachings of Tomes Only Relate to Transient Expression of a Transgene

The Action asserts that Tomes "teaches and claims a particle bombardment-mediated method for the production of fertile transgenic maize plants…wherein the transformation of embryos or embryogenic cells results in stable transformation and the transmission of the introduced transgene to progeny plants and seed…" [Action, page 4]. Applicants respectfully submit that the cited disclosure of Tomes does not render the present invention obvious, because the Tomes specification shows only transient expression of a transgene and would in no way provide a reasonable expectation of practicing the presently claimed invention.

i) Only Transiently Transformed Corn Cell Lines Are Provided By Tomes

As an initial matter, Applicants briefly summarize the process for producing transgenic plants at the priority date of the present application. Briefly, a DNA construct designed for expression of a heterologous gene in a plant (*e.g.* corn) is made, and introduced into a plant ***cell***. The construct may have a screenable or selectable marker or reporter gene so that expression of a gene or genes may be followed. The cells may be non-regenerable (*e.g.* "BMS" corn line) or regenerable, and is typically a non-differentiated cell such as a callus cell, if a fully (*i.e.* non-chimeric) transformed plant is eventually desired. Upon introduction into a plant cell, the construct is not initially integrated into the genome, but may express "transiently" for a period. If the construct is not integrated into the genome of the host cell, expression of the marker gene is eventually lost (*i.e.* declines to background), and the construct and encoded product is not found in cells or plants derived from any initially transformed cells. Transient expression of a DNA construct in plant cells is often studied to demonstrate that a construct is capable of transient expression generally, but does not necessarily result in production of a (stably) transformed plant, let alone a fertile plant, let alone a fertile plant capable of passing on the introduced genetic trait to a subsequent generation.

Regeneration of transformed (regenerable) plant cells to form transformed plants is an additional necessary step in the process to obtain transformed fertile plants. Selection for the presence of the heterologous DNA construct was necessary to allow identification of the very small percentage of transformed plant cells, themselves a small percentage of the total number of cells that were subjected to introduction of the DNA construct, that may have "stably" integrated the construct into their genome, such that it can be expressed and is thus found in cells derived from the initially transformed cell, for instance via cell culture. The initially transformed cell and

cells derived from the cell continue to grow, divide, and differentiate under appropriate culture conditions. If a selective agent is present, non-transformed cells are inhibited in growth, and transformed cells predominate in the cell culture, eventually giving rise to regenerated plants. Application of selective pressure increases the likelihood that the germline tissue of a regenerating plant comprises the DNA construct, allowing it to potentially pass the introduced nucleic acid sequence to a subsequent generation of plants. However, such plants may or may not be fertile, and if fertile, may or may not pass the heterologous construct on in their germline.

Accordingly, the numerous steps involved, including preparation of a DNA construct; introduction of a construct into a cell; identification/selection of a ***stably transformed*** cell; cell culture to allow for regeneration of a differentiated stably transformed plant from an initially transformed cell that has stably integrated a transgene; production of a transformed plant; and production, by the plant, of gametes that comprise the introduced DNA construct, are all necessary in achieving the presently claimed invention, as is the expression of the DNA construct to cause the plant to exhibit phenotypic characteristics that render it identifiable over the corresponding untransformed maize plant. Tomes does not teach all of these steps for corn plants nor would it provide a reasonable expectation of success to one of skill in the art as of the priority date in arriving at the invention. In particular Tomes does not teach or demonstrate any step for identifying and selecting a stably transformed cell after initially introducing a construct into a cell, or any step that would lead to a stably transformed cell, let alone stably transformed plant, let alone a stably transformed fertile plant or progeny thereof, subsequent to that step of initially introducing a construct into a cell. Indeed, as discussed below, the Tomes inventors conceded that they had not put together such necessary steps for producing transgenic corn plants. Instead, Tomes only lays out some initial steps, such as assembling a DNA construct that

may be expressed in a corn plant ***cell***, and introducing the construct into a corn plant cell such that it is expressed ***transiently*** for up to 12 days. Tomes does not demonstrate ***stable integration*** of their construct into a plant genome. Neither does Tomes teach how to regenerate a stably transformed cell into a corn plant that is fertile and that can pass the introduced DNA construct to a subsequent generation. There is therefore no basis to suggest one of skill in the art would have any reasonable expectation that a fertile, transgenic maize plant could be produced, the genome of which has been augmented with a gene encoding a grain composition trait comprising a fatty acid desaturase gene such that the transgenic plant exhibits one or more phenotypic characteristics that render it identifiable over the corresponding untransformed maize plant, and wherein said gene is transmittable through normal sexual reproduction of the transgenic maize plant to subsequent generation plants.

The Action fails to identify where Tomes or the art generally provides fertile transgenic corn plants, let alone any fertile transgenic corn plants augmented with a grain composition trait. Although the Action apparently understands Tomes to show that such corn plants are provided, a careful reading of Tomes clearly indicates that this is inaccurate, and has no support in the Tomes application ***as filed***. That is, no fertile transgenic corn plants are provided by Tomes, let alone any of a subsequent generation, nor are methods provided that would provide a skilled artisan with a reasonable expectation of success in producing such plants. For instance, assays of regenerable corn cell lines 3-86-17 and 13-217 were only performed (on cultured cells) up to 12 days post-transformation (Tomes, column 8, lines 54-65), and the assay results are explicitly described as relating to cell lines (Tomes, column 8, lines 64-65), and not to plants, seeds, or progeny plants of a subsequent generation that are stably transformed. No data is even provided by Tomes clearly demonstrating stable transformation of regenerable corn cells, which could

possibly have been regenerated into stably transformed corn plants. Indeed, stably transformed cells, let alone stably transformed regenerable cells, are not even provided. Rather, the Specification only discloses ***transiently transformed corn cells***, and there is no indication in the Tomes patent as filed that a fertile transgenic corn plant of a subsequent generation could have been produced, or how to achieve such a result.

ii) The Tomes Inventors Concede That They Had Not Produced Stably Transformed Corn Cells In The Experiments Described.

Applicants further respectfully bring to the Examiner's attention two articles by multiple inventors of Tomes (Weissinger *et al.,* including Tomes ("Maize Transformation via Microprojectile Bombardment", pp. 21-25 in Current Communications in Molecular Biology, ed. Fraley *et al.* (1988))), "Weissinger"; cited in IDS, considered by examiner May 7, 2004; and Klein *et al., PNAS* 85:4305-4309, 1988; "Klein"; **copy provided herewith**). These two publications describe experiments that are apparently identical to those described in Tomes, in Examples 1-4. In particular, cells of the non-regenerable "BMS" corn cell line, and the alleged regenerable corn cell lines 3-86-17 and 13-217 were subjected to particle bombardment with pCaMVCN and pCaMV$_1$CN. Indeed, some of the experiments described in these publications are clearly ***the same*** as those described in the cited Tomes patent.

For instance, bombardment of BMS cells with a plasmid comprising a CAT reporter gene is described as resulting in expression of CAT activity as high as 57 units per gram of soluble protein, while bombardment of regenerable 3-86-17 and 13-217 cell lines is stated to have yielded expression of only 3 units per gram of soluble protein (Weissinger, page 23). These enzyme activity levels may be compared with those stated in Tomes, for instance at Tables 1-2.

Likewise the results of Klein (*e.g.* pp. 4307-4308) may be compared to Tomes, columns 8-10. For instance, Klein Table 1 may be compared with Tomes Table 1, Klein Table 2 may be compared with Tomes Table 3, Klein Table 3 may be compared with Tomes Table 4, and Klein Table 4 may be compared with Tomes Table 5. Apart from a number of typographic errors, the ***same experiments are evidently being described in Tomes, Klein, and Weissinger***. Only Tomes Table 2 is not explicitly included within Klein or Weissinger. However, Table 2 of Tomes only relates to transient transformation of corn cells, between 96 hours and 12 days post-bombardment (*e.g.* Tomes, column 8, lines 64-66), and the result is mentioned by Weissinger at page 23, end of first full paragraph ("…levels of approximately 3 units per gram..."), and only within the context of ***transient*** transformation of corn cells, as noted below regarding page 24 of Weissinger. Example 4 of Tomes describes additional transformations of 3-86-17 cells, including with β-glucuronidase reporter gene, but again, the reporter enzyme activity was assessed only in transient assays only 3 days post-bombardment, and no ***stable*** transformation, let alone production of any transgenic plants, whether fertile or infertile, is described.

Applicants note that Weissinger *et al.*, at page 24, middle of first paragraph, ***explicitly concede that stable transformation even of corn cells had not been achieved.*** In particular:

> "…Embryogenic (3-86-17 and 3-217 [sic]) suspension cultures bombarded with this mixture were found to produce levels of CAT enzyme activity 12 days after plating on selective levels of hygromycin…**Although these cultures have not yet yielded proven stable transformants**…"[p.23, emphasis added].

Applicants also bring the Examiner's attention to the last sentences of the Weissinger article, which states that:

> "As it is now engendered, microprojectile bombardment is a rapid and efficient technique that offers a useful alternative to electroporation **for transient gene**

expression studies in maize. Recovery of transformed maize plants produced by means of this unusual technology has become a **real possibility** [pp. 24-25, emphasis added]".

Thus, it is clear from inventors' own words that their experiments relate specifically to ***transient gene expression***; that stably transformed corn cells, let alone plants, ***remained only a possibility*** that had not been achieved**;** and that ***activity of a reporter enzyme even 12 days post-bombardment was not an indication of stable transformation***. Klein also concedes that "(n)umerous bombardment and culture variables remain to be determined…" (*e.g.* Klein, page 4309, left column, 2nd to last paragraph), and their article concludes by focusing on the use of their methods for transient expression studies, such as tissue-specific transient expression (*i.e.* in differentiated tissues such as leaves). Nowhere does Klein indicate how their methods may be used to create transgenic corn plants that can pass a transgene to a subsequent generation, or transgenic progeny of such plants, or that such plants could express a transgene to exhibit a phenotypic characteristic rendering it identifiable over the corresponding untransformed maize plant.

Applicants note that neither Tomes nor Weissinger nor Klein describe assays carried out later than 12 days post-transformation, while they explicitly concede that stable transformation had not been shown. Transient transformation assay data, as acknowledged by the Tomes inventors, is quite distinct and not predictive that stable transformation will follow. It simply could not be predicted or assumed based on the teachings of Tomes that transient reporter gene expression will lead to transgenic corn plants, let alone fertile transgenic corn plants expressing a grain composition trait. In total, the data (and lack thereof) provided, as well as the explicit concession by the authors that stable transformation had not been demonstrated, clearly indicates

that Tomes alone or in combination simply does not provide a reasonable expectation that stably transformed corn plants could be produced expressing a gene encoding a grain composition trait comprising a fatty acid desaturase gene such that the transgenic plant exhibits a phenotypic characteristics rendering it identifiable over the corresponding untransformed corn plant.

iii) Tomes is Not Properly Applied.

Notwithstanding the assertion in the Action at page 4, first full paragraph, Applicants do not concede that Tomes *et al.* teach a method for the production of fertile transgenic corn plants comprising a foreign transgene of interest. Further, in view of the concession by Tomes inventors that their experiments resulted only in production of ***transiently transformed corn cells*****,** and not even in stably transformed corn cells that would be necessary to eventually achieve fertile transgenic corn plants, Applicants respectfully submit that Tomes fails to disclose an actual fertile, transgenic maize plant capable of transmitting an introduced gene through normal sexual reproduction of the transgenic maize plant to subsequent generation plants and thus all elements of the claims are not found in the cited references. Because Tomes provides no teachings as to how to achieve stably transformed fertile transgenic corn plants and expressing an introduced gene and subsequent progeny generations thereof, and the other references cited fail to remedy this deficiency, the Action has failed to show that all elements of the claims are in the prior art.

As noted above, Tomes does not describe how to stably transform a corn plant cell and how to select for and regenerate a stably transformed transgenic plant from a stably transformed cell. The Weissinger and Klein articles, co-authored in total by all of the inventors of Tomes, shows that the experiments disclosed for instance in Example 1 of Tomes did ***not*** result in

production of stably transformed corn cells, let alone plants. In particular, the Tomes specification contains no additional data contradicting the admissions of the Weissinger publication, which is authored by three of the four co-inventors of Tomes. As such, by the clear admission of most if not all of the Tomes inventors, Tomes does not render the present invention obvious, regardless of whether U.S. 6,258,999 of Tomes is presumed valid, because the Tomes specification is ***inoperable*** with respect to the presently claimed invention. Additionally, Applicants submit that, since Tomes is inoperable for achieving fertile transgenic corn plants, there would have been ***no expectation of success*** in combining Tomes with either Martin or Thompson, especially in view of the Weissinger and Klein articles discussed above, in which the Tomes inventors cumulatively concede that their studies did not result in stably transformed corn cells. Thus, the Action has also failed to show that all elements of the claimed invention are found in the cited art.

2. Rejection in view of Tomes *et al.* and further in view of Martin *et al.*, and Thompson *et al.*

The Action cites Martin as teaching the isolation of a yeast gene encoding a fatty acid desaturase and suggesting that maize may be transformed with a gene for a fatty acid desaturase. The additional references fail to overcome the deficiencies of Tomes as mentioned above.

For example, the Action cites Martin at column 2, lines 67-68; column 3, lines, 1-41; column 4 at lines 21-29, column 5, lines 2-8, columns 11-12, and Table 2 in support of the rejection. However, a review of the Martin Specification shows that only column 4, lines 21-29, and column 5, lines 2-8, as well as column 10, line 57, even mention introduction of a fatty acid modifying enzyme into a plant cell. Even at these locations, the teachings of Martin are entirely

cursory, and as described below, would not give a skilled artisan any ***expectation of success***, as of the August 25, 1993 priority date, to achieve production of plants with an identifiable grain composition trait comprising a fatty acid desaturase gene.

Nowhere does Martin acknowledge that fatty acid synthesis in yeast diverges in numerous fundamental aspects from that found in plants. For instance, *de novo* fatty acid synthesis in yeast and mammals occurs in the cytoplasm, and is accomplished by the so-called Type I fatty acid synthase, a membrane associated (ER) multi-enzyme complex (*e.g.* Smith, *FASEB J.* 8:1248-1259, 1994). However, in plants, the site of such synthesis is the chloroplast, and is accomplished by a series of single enzyme polypeptides, including one termed a "Type II fatty acid synthase" (*e.g.* Browse and Somerville, *Ann. Rev. Plant Phys. Plant Molec. Biol.* 42:476-506, 1991). Unlike the animal/yeast enzymes, plant d9 desaturases are soluble proteins found in the chloroplast stroma. Further, the substrate for the plant d9 desaturase is 18:0-Acyl Carrier Protein (ACP) which is not found in yeast or animals; instead the animal/yeast enzymes use acyl-CoA as substrate/co-factor. Due to these fundamental biochemical differences in fatty acid desaturation between animal/yeast on the one hand and plants on the other, even if a yeast d9 desaturase were expressed in a corn cell, assuming it would have been stable, it would have been expected to localize to the cytosol and ER membranes, not the chloroplast, and one of skill in the relevant arts would have had **no expectation** that it would function correctly in a plant cell or would lead to enhanced oil content.

Further, at column 4, lines 21-29, Martin cursorily discusses introducing the yeast d9 desaturase into other organisms, or isolating and using "similar" desaturase genes from other organisms including crop plants. However, the yeast d9 desaturase of Martin is similar to the rat desaturase found in liver membranes (Martin, column 3, line 46), and is clearly a Type I

synthase. Martin *et al.* are apparently unaware that there is essentially no sequence similarity between plant and animal/yeast fatty acid desaturases (e.g. Browse and Somerville, above). Yeast and plant d9 desaturases have evolved independently. Thus, Martin does not provide teachings, at least for plants, that would allow a skilled artisan to expect success in also applying any teachings of Tomes and Thompson, such as they are being asserted, to produce plant cells and plants with a grain composition trait such as modified fatty acid content.

Applicants also bring to the Examiner's attention a reference by Polashock *et al.* (including Martin; [*Pl. Physiol.* 100:894-901, 1992]), published ***4 years*** after the cited Martin patent's filing date, which relates to expression of a yeast d-9 fatty acid desaturase in *Nicotiana tabacum*, and yet is unable to demonstrate clear trends of changes in seed fatty acid composition by expression of the yeast enzyme. Although this reference describes some activity of the yeast enzyme in plants, it also extensively discusses the differences in animal versus plant fatty acid synthesis, as outlined above. At Table I, Applicants note that the Polashock reference shows that their transgenic plants lacked significant changes in seed oil composition (*e.g.* relative proportions of various fatty acids), while overall seed oil levels are not even discussed. The reference also repeatedly states that no phenotypic differences were observed between wild type and transgenic plants.

Importantly, in Table I, the reported changes in relative amounts (mol %) of the various fatty acids in the different tested tissues shows that changes in seeds were distinct from those in other tested tissues such as leaves, stems, roots, and ovaries. For instance, when comparing wild type and transgenic plants for fatty acid distribution, 16:0 fatty acid (palmitic acid, a saturated fatty acid) in seeds decreased only slightly while in the other tested tissues it decreased significantly. Likewise, the proportion of 16:1 (*e.g.* palmitoleic acid, an unsaturated fatty acid)

increased only modestly in seeds, but to a much greater extent in the other tested tissues; and 18:1 fatty acids (*e.g.* oleic acid, an unsaturated fatty acid) even ***decreased*** in seeds while increasing in other tested tissues. Taken together, these results from the Martin laboratory for seed fatty acid synthesis show no clear or consistent trend upon expression of the yeast enzyme in tobacco. Thus, one of skill would not have routinely expected that use of a yeast enzyme in oil seed plants such as corn would lead to seed with useful alteration of fatty acid levels (*i.e.* a grain composition trait), even four years after the filing date of the Martin patent. Indeed, if anything, the results of Polashock and Martin, *et al.* **teach away** from use of, at least, a yeast enzyme to alter a corn plant grain composition strait such as altered seed oil. This is underscored by the fact that the cited art fails to provide a reasonable expectation of success in expressing any transgene in corn.

The failings in the cited art are greatly contrasted by the teachings of the current specification which, for example, indicates after Table 8 that fertile plants were obtained by the inventors from 267 different transgenic lines produced. In Table 9, the specification describes the creation of numerous transgenic maize plants with a variety of different genes using many different regulatory elements. For example, the table shows the creation of R0 transgenic plants and confirmation of transgene expression in these plants and progeny using the following genes: a *uidA* reporter gene, a *bar* selectable marker gene conferring herbicide tolerance, a *hyg* gene conferring resistance to hygromycin, an *aroA* gene conferring tolerance to the herbicide glyphosate, a *Bacillus thuringiensis* endotoxin gene, and a Z10 altered seed storage protein. The Table further shows that transgenic maize callus was obtained transformed with a C1 anthocyanin pigmentation gene, a *lux* luciferase reporter gene, potato and tomato *pinII* proteinase

inhibitor genes conferring insect resistance, an *mtlD* protein conferring enhanced stress resistance and a *deh* gene conferring resistance to dalapon herbicide.

In contrast to the specification teaching, the cited references simply provide no reasonable expectation that ***any foreign gene*** could be introduced and stably expressed in fertile transgenic maize, let alone a plant augmented with a gene encoding a grain composition trait comprising a fatty acid desaturase gene such that the transgenic plant exhibits at least one phenotypic characteristic rendering it identifiable over a corresponding untransformed maize plant and wherein the gene is transmittable through normal sexual reproduction of the transgenic maize plant to subsequent generation plants.

The Action further asserts that Thompson *et al.* (U.S. Patent 6,177,677) teach isolation of a safflower gene encoding stearoyl-ACP desaturase, which is a synonym for delta-9-[fatty acid] desaturase, as well as production of DNA constructs comprising the desaturase gene, for expression in transgenic plants. Thompson is further asserted to "suggest" transformation of corn to produce corn oil with decreased levels of saturated fatty acids (*e.g.* column 1, lines 17-35; column 6, line 44, to column 8, line 9; column 8, lines 43-49; column 9, lines 16-19 and 28-30; column 14, line 25, through column 16, line 37; column 17, line 55, through column 25, line 44; and claims 1-2, 5, 13, and 22-23). Applicants respectfully traverse.

Applicants note that Thompson mentions the term "corn" only twice in the entire Specification, and only in passing. For example, at column 5, line 67, the possibility of isolating a corn stearoyl-ACP desaturase is briefly touched upon. However, this does not teach how one might express such a gene in a corn plant to successfully achieve a modified grain composition trait. At column 9, line 19, "corn" is mentioned among several other crops grown for production of vegetable oils, however with no teachings specific to corn. Importantly as well, none of the

other crops listed there is a monocot. Again, there are no teachings that would lead a skilled artisan to expect that expression of a fatty acid desaturase transgene in a corn plant would successfully achieve a modified grain composition trait.

Since Thompson includes essentially no teachings relating specifically to corn (maize), the present rejection in view of Tomes, Thompson, and Martin, clearly represents hindsight reasoning, and the cited references, understood together as well as separately, are mischaracterized. A skilled artisan at the filing date of the present application in possession of the cited art simply would not have expected that expression of a fatty acid synthesis-related transgene would successfully result in a modified grain composition trait in transgenic corn. Barring impermissible hindsight reasoning the presently claimed invention therefore represents an ***unexpected and non-obvious result***. Withdrawal of the obviousness rejection is therefore respectfully requested.

D. Conclusion

The examiner is invited to contact the undersigned with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,

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